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Development and Relative Validation of a Food Frequency Questionnaire to Assess the Intake of Non-Nutritive Sweeteners in Pregnant Women in Santiago de Chile: A Pilot Study

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Development and Relative Validation of a Food Frequency Questionnaire to Assess Non-Nutritive Sweeteners Intake among Pregnant Women in Santiago, Chile: A Pilot Study

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Abstract: Studies on the effects of non-nutritive sweeteners (NNSs) among pregnant women are scarce and have produced mixed results. One of the major challenges is to assess accurately NNSs intake, especially in countries that have implemented policies to prevent obesity and where many foods and beverages have been progressively reformulated to partially or totally replace sugar by NNSs. This study aimed to develop and assess the relative validity of a food frequency questionnaire (FFQ) for use in pregnant women. We developed an FFQ to examine the intake of seven NNSs (acesulfame-k, aspartame, cyclamate, saccharin, sucralose, steviol glycosides and D-tagatose). This questionnaire was piloted in 29 pregnant women (median age= 31.2y; (25th- 75th percentile: 26.9-34.7) to assess NNSs intake over the previous month, compared to a 3-day dietary records (3-DR). The validity of this dietary method was assessed using Spearman´s correlation coefficient, Lin´s concordance correlation coefficient (CCC) and Bland-Altman plots. Spearman correlations between FFQ of NNSs and 3-DR ranged from 0.50 for acesulfame K to 0.83 for saccharin. CCC ranged between 0.22 to 0.66. The Bland-Altman plots showed an overestimation of saccharin, sucralose and steviol glycosides intake by the FFQ of NNSs compared with 3-DR, and an underestimation of acesulfame K and aspartame. Overall, the NNSs most frequently consumed were sucralose and none of the participants exceeded the acceptable daily intake for any of the NNSs evaluated. The FFQ of NNSs seems to be reasonably valid in the assessment of NNSs among pregnant women.

Keywords: non-nutritive sweeteners; food frequency questionnaire; survey validation; pregnant women

1. Introduction

Non-nutritive sweeteners (NNSs) are food additives that are used in a variety of foods and beverages to reduce their energy and sugar density, or as tabletop sweeteners [1,2]. NNSs have been associated with a wide range of adverse health effects, some particularly related to cancer risk [3] and more recently, with alterations of food intake, mood, blood pressure, body weight, abdominal obesity, diabetes, and neurodegenerative diseases such as dementia [4–7]. Accordingly, the World Health Organisation (WHO) recently stated that the consumption of NNSs should be limited due to their potential long-term undesirable effects [8]. It is clear that these compounds are not inert in the body, but can affect the consumer's physiology through mechanisms that may explain some of their

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adverse metabolic effects. RMI studies in individuals exposed to sucrose or sucralose indicate, for example, that our brains can differentiate ENNs from the caloric sweetener [9]. ENNs would induce a dissociation between the sweet taste perceived in the mouth and the absence of calories associated with the food containing them, a phenomenon that could lead to a compensatory increase in appetite, energy intake and weight gain. In addition, they may stimulate sweet taste receptors in intestinal cells, increasing the presence of glucose transporters and postprandial glucose absorption [10]. ENNs also modifies the composition and function of our gut microbiota, increasing its virulence and promoting the development of glucose intolerance and liver inflammation [11].

Nonetheless, many studies have yielded conflicting results, and there is not supported by well-performed systematic reviews and meta-analyses of both prospective and randomized control trials, so the dangers of consuming NNSs are still debated [12]. Some decades ago, relatively few ingredients were available to sweeten foods and beverages. Since then, the situation has dramatically changed and dozens of synthetic and natural compounds can be used to replace sugar in food products [1,2,13]. Therefore, NNSs consumption has increased in the recent years among adults and children [13–16]. In Chile, the Food Labeling and Advertising law 20.606 establishes limits for the content of sugars, sodium, saturated fats, and total energy in processed foods. Those exceeding the limits for one or more critical nutrient must exhibit a front-of-package warning label consisting of a black hexagon stating in white letters "High in …," with the identity of the corresponding critical nutrient [17]. Since law came into force, many foods and beverages have been progressively reformulated to partially or totally replace sugar by NNSs [18,19]. Accordingly, the proportion of processed foods containing NNSs has considerably grown in the last years and we recently shown that 55.5% of the food products available in Chilean supermarkets contained NSS, i.e. much more than in other countries [20]. In addition, about 80 tabletop NNSs are available in the local market, which also contribute to the consumption of these additives [20].

A particular interest regarding NNSs intake is their possible effect on fetal development *in utero* and their use during pivotal periods such as pregnancy and lactation, because of the possibility of metabolic alterations or other consequences in both the mother and offspring [2,13,21,22]. Previously, two cohort studies investigated the relationship between artificially sweetened soft drink intake by pregnant women and preterm delivery in Norway and Denmark showing an increase on the risk of preterm birth [23,24]. In addition, Maslova et al. found a positive association between the risk of developing asthma and allergic rhinitis during childhood and the maternal consumption of artificially sweetened soft drink during pregnancy, compared to non-consumers [25]. Nonetheless, in another cohort study from England, the intake of artificially sweetened beverages did not influence preterm delivery [26]. Likewise, a meta-analysis that pooled the main findings of the Norwegian and Danish studies previously mentioned did not find an impact on preterm delivery as compared between sugar beverages and artificially sweetened beverages consumers [27]. These contradictory results might be in part due to different approaches used to measure NNSs intake. Usually, consumption of artificially sweetened beverages has been used as a proxy for NNSs intake [28], but the validity of this approach decreases when the variety of NSS-containing foods increases, as in Chile.

There is therefore an urgent need to develop valid, efficient, and cost-effective instruments to accurately assess NNSs consumption. Traditionally, Food Frequency Questionnaires (FFQs) have been the preferred method used in epidemiological studies for assessing habitual dietary intake over long periods and in large samples due to lower participant burden, less time-consuming and less intrusive for participants [29–31]. It is important to evaluate the FFQs in the target population before its implementation, and to assess its validity comparing with an appropriate reference dietary method [32]. FFQs are commonly validated through 24 hour recalls or dietary records [33]. To our knowledge, there are not studies that assessed accurately NNSs intake among pregnant women, considering the variation in NNSs content due to portion sizes, brands and flavors and especially in countries that have implemented regulations to prevent obesity and many foods and beverages have been progressively reformulated to partially or totally replace sugar by NNSs [34].

Therefore, this pilot study aimed to develop and assess the relative validity of an FFQ to evaluate the intake of seven NNSs (acesulfame-k, aspartame, cyclamate, saccharin, sucralose, steviol glycosides and D-tagatose) among pregnant women living in Santiago, Chile. The seven NNSs were selected based on the availability of analytical data and because they were the most common NNSs in foods and beverages in the Chilean market at the time of data collection [20].

2. Material and Methods

2.1. Study Design

This work was undertaken as part of the study "Determination of fetal and infant exposure to non-caloric sweeteners study" whose aim is to determine NNSs concentrations (sucralose, saccharin, acesulfame-k, cyclamate and steviol) in amniotic liquid and breastmilk and correlate them with maternal NNSs intake, in order to evaluate fetal/infant exposure to these compounds. In addition, it aims to determine whether a higher intake and concentration of NNSs influence sweet taste threshold and preference as well as weight gain during the first year in their offspring (https://clinicaltrials.gov/ct2/show/NCT03972176). The current FFQ of NNSs validation study was conducted as an initial step prior to subject recruitment and data collection for this study. Accordingly, subjects included in the validation step were not participants of the main study.

A convenience sample of pregnant women were recruited from Santiago Metropolitan Area from May 2019 to September 2019, using flyers, advertisements, social media posts, and word of mouth. These mothers were attended in Primary Health Care Centers from the surrounding neighborhood area to Faculty of Medicine at University of Chile. Initially, 75 pregnant women were contacted, and the following eligibility criteria were applied: singleton pregnancy, being in the last trimester of pregnancy, ≥ 18 years of age, fluent in Spanish, without diagnosis of diabetes prior to pregnancy and more than two years of residence in Chile. Forty-five women met these criteria and were invited to participate a face to face interview. These who agreed to participate signed an informed consent, completed a short sociodemographic questionnaire, answered some questions regarding their previous pregnancies and current pregnancy and self-reported their current weight. Trained nutritionists applied the FFQ of NNSs and upon completion of the face to face interview, women were provided with a three-day dietary record to be returned once completed to the nutritionist within the following 7 days. The final sample with comprehensive information on the FFQ of NNSS and 3-day dietary records included 29 pregnant women. The Ethics Committee of Faculty of Medicine at University of Chile approved the study.

2.2. Food Frequency Questionnaire

The development of the FFQ of NNSs was divided into two phases: design (phase 1) and piloted validation (phase 2). In phase 1, a food and beverage list was created specifically to capture NNSs intake over previous month. The NNSs content was obtained after trained personnel carried out a comprehensive search visiting nineteen supermarkets and gathering information from thirteen web pages from foods and beverages and tabletop NNSs. The information included ingredients, concentrations per serving size, per 100 g, brands and flavors of all foods and beverages available in the Chilean market at the time of data collection [16]. Overall, a total of 815 foods and beverages items were found that contained at least one of these NNSs: acesulfame-k, aspartame, cyclamate, saccharin, sucralose, steviol glycosides and D-tagatose, and were organized in six food groups: i) dairy products; ii) cereal products; iii) processed fruits; iv) non-alcoholic beverages; v) sweets and other desserts and vi) tabletop NNSs. All the information was entered in an Excel spreadsheet to assign the values for NNSs content in milligrams (mg) to all NNSs products found per serving size and per 100 g. Furthermore, to stimulate participants´ memory, a photographic atlas of all the products with NNSs (including specific brands and flavors) ordered by food groups was developed, which also contained images of dishes, glasses, and tablespoons to estimate the size of the ingested portion. The food database and atlas were regularly updated in the course of the study in order to integrate the new NNS-containing products launched on the market and, eventually, to eliminate those that were withdrawn. A semi quantitative questionnaire with open-ended options was used to prevent loss of information and misclassification [35] (see Figure 1). Serving sizes were estimated based on amounts commonly purchased or eaten according to the National Chilean Dietary Survey (Encuesta Nacional de Consumo Alimentario, ENCA) [36]. A team of nutritionists reviewed and confirmed the content validity of the questionnaire.

Food group	How often?				Medium serving	Your serving size	Comments
	Day	Week	Month	Rarely/ never			
Dairy products							
Flavored milk (strawberry)					1 or 200 ml		
Flavored milk (vanilla)					1 or 200 ml		
Flavored milk (chocolate)					1 or 200 ml		

Figure 1. Example of the semiquantitative food frequency questionnaire with open-ended options used in this pilot study.

In phase 2, data was collected through the administration of written questionnaires during the face to face interview. A trained nutritionist asked participants whether they had consumed a particular food or beverage in the previous month. Participants who answered affirmatively were asked how often it was consumed as well as the serving size in household measures. For each NNSs considered in this study, the amount of daily NNSs intake was calculated as follows: Daily NNSs intake (mg/day) = [Number of daily serving sizes X Frequency intake monthly X Serving size amount (g or mL) X Conc of each NNSs in the food matrix (mg/100 g)]/30. Afterwards, we determined the daily intake per kilogram of body weight of each NNSs by dividing the daily intake previously calculated by the self-reported body weight (mg/kg bw). Finally, such values were summed up to obtain the total daily NNSs intake.

2.3. Three-Day Dietary Records

A three-day dietary record (3-DR) was used to assess the NNS consumption of all participants over a week. After the face to face interview, participants were instructed to provide as accurately as possible all foods consumed and highlight those containing NNSs on two weekdays and one weekend day. Records requested information on food and beverage items (with brand names and flavor, where possible), portion sizes in household measures, time and location of meals. Food records were reviewed, and participants were contacted by the nutritionist to clarify missing or incomplete information. The results were entered into an Excel file to convert all the food/beverage items into a daily NNSs intake and the daily intake per kg of body weight. Intakes from three-day dietary records were averaged across the three days. The Food Processor software 10.9 COPYRIGHT © ESHA RESEARCH, INC., 2011, and the Composition Table of the Chilean Foods, as well as information provided by the manufacturers were used to estimate calorie and macronutrient intake.

2.4. Statistical analyses

Statistical analyses were performed with Stata SE version 14 (StataCorp LLC, College Station, TX, USA). Data were checked for normality using Shapiro-Wilk test and histograms. This analysis determined that continuous variables were not normally distributed, therefore, we reported continuous variables as medians and 25th, 75th percentile, while or the categorical variables are presented as frequencies and percentages. We described the daily intake of NNSs (mg/day) and the daily NNSs intake per kg of body weight per consumer and we compared with the Acceptable Daily Intake of NNSs (ADI) defined by the European Food Safety Authority (EFSA) [4]. The validity of the FFQ of NNSs was assessed by comparing each and total NNSs intake measured by the FFQ of NNSs against those measured with the average of the 3-DR. Spearman correlation was used to identify the relationship between NNSs intake obtained from FFQ of NNSs and 3-DR. We also analyzed the data

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using the concordance correlation coefficient by Lin (CCC). It is the correlation between the two measurements that fall on the 45° line through the origin. The CCC ranges from 0, indicating no substantial agreement, up to 1, representing perfect concordance [37]. We preferred CCC instead of Intraclass Correlation Coefficient (ICC) or paired t- test, because there are some drawbacks with all these methods [37–39]. In addition, a sample size calculation was performed for Bland-Altman analysis of agreement [40]. Based on the mean difference between FFQ and Dietary Records as well as standard deviation of the differences from Myers at al. [28], a maximum allowed difference of 80 mg among total NNSs, and an α level of 0.05 and a power of 0.8, a sample size of 25 was required. The Bland-Altman plot [41] was used to assess graphically the agreement between both instruments using mean difference and 95% limits of agreement (LoA). The analysis of both the CCC and the Bland-Altman plots were conducted using the Stata "concord" command. The results were considered statistically significant at a 0.05 level (two-tailed).

3. Results

3.1. Descriptive Statistics

A total of 45 women were recruited, but only 29 completed the study. Half of the participants were 31.2 years (25^{th} -75^{th} percentile: 26.9-34.7), 69% were primiparous and with a median of 30 gestational weeks. More than two thirds of the sample were Chilean, with the remainder being of other nationalities, mainly Venezuelan, Peruvian, Colombian, and Ecuadorian. Their average daily energy intake was 1873 ±416 kcal. More than half (52.7%) of the total calorie intake came from carbohydrates, while total fats and proteins contributed 32.3% and 15%, respectively. More than 65% of the participants had a high level of education and 80% were in the labor force (Table 1).

Table 1. Characteristics of participants in the pilot study n= 29.

Characteristics	25^{th}-75^{th} percentile or %
Age (years) median (IQR)[1]	31.2 (26.9-34.7)
Gestational weeks median (IQR)[1]	30.0 (28.0- 32.0)
Parity n (%)	20 (69.0)
0	9 (31.0)
≥ 1	60.0 (55.0- 66.0)
Pregestational weight median (IQR)[1]	68.0 (63.0-73.0)
Current weight median (IQR)[1]	3 (10.3)
Medical history n (%)	3 (10.3)
Gestational Diabetes	1 (3.4)
Hypothyroidism	20 (69.0)
High Blood Pressure	9 (31.0)
Nationality n (%)	
Chilean	7 (24.1)
Other	3 (10.4)
Education n (%)	19 (65.5)
High School	
Vocational training	23 (79.3)
College or more	6 (20.7)
Employment n (%)	1873 ± 416
Yes	75.5 ± 26.3
No	245.3 ± 64.0
Diet	70.0 ± 7.5
Total energy intake (kcal/d). Mean ± SD[2]	
Protein intake (g/d). Mean ± SD[2]	

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Carbohydrate intake (g/d). Mean ± SD[2]	
Fat intake (g/d). Mean ± SD[2]	

[1] IQR: Interquartile range; [2] SD: Standard deviation.

Of the 29 participants, 93.1% (n=27) used NNSs daily in the FFQ, while 82.8% (n=24) used NNSs in the 3-DR. The three NNSs most frequently consumed were sucralose, followed by steviol glycosides and acesulfame K. Overall, the median daily NNSs intake of sucralose, steviol glycosides and D-tagatose according to the FFQ were higher than the intake reported through the 3-DR. The median daily NNSs intake from the FFQ of NNSs was 1.49 mg/kg bw (25th-75th percentile: 0.73- 3.37) vs 1.28 mg/kg bw (25th-75th percentile: 0.62- 2.48) daily NNSs intake reported from the 3-DR. When compared with ADI, none of the participants exceeded the acceptable daily intake for any of the NNSs evaluated (Table 2).

Table 2. Daily NNSs intake (mg/day) and daily NNSs intake per kilogram of body weight (mg/ kg bw) from the FFQ of NNSs and the average of 3-DR and comparison with the Acceptable Daily Intake (ADI) by EFSA among pregnant women reporting any consumption of NNNs.

NNS Type	n[a]	FFQ of Daily NNS intake (mg/day)	FFQ of daily NNS intake (mg/ kg bw)	n[a]	3-DR of Daily NNS intake (mg/day)	3-DR of daily NNS intake (mg/ kg bw)	ADI defined by EFSA (mg/kg bw)
		Median (25th-75th percentile)	Median (25th-75th percentile)		Median (25th-75th percentile)	Median (25th-75th percentile)	
Acesulfame K	23	20.9 (7.0- 69.7)	0.38 (0.11- 1.00)	15	40.5 (32.0- 71.6)	0.65 (0.45- 1.16)	9
Aspartame	19	59.1 (24.0- 84.6)	0.38 (0.02- 0.86)	14	84.0 (58.5- 120.0)	0.40 (0.01- 0.93)	40
Cyclamate	1	3.1[b]	0.06[b]	1	38.5[b]	0.71[b]	7
Saccharin	3	1.1 (0.01- 79.2)	0.02 (0.00- 1.28)	2	22.3 (13.0- 31.5)	0.37 (0.24- 0.51)	5
Sucralose	27	24.8 (14.6- 60.6)	0.38 (0.20- 0.94)	23	22.4 (9.4- 46.5)	0.33 (0.17- 0.75)	15
Steviol glycosides	25	22.0 (4.2- 31.2)	0.31 (0.06- 0.49)	18	21.6 (7.8- 41.3)	0.31 (0.12- 0.62)	4
D-Tagatose	1	3.5[b]	0.05[b]	1	2.7	0.04[b]	not specified
Total NNS intake	27	93.0 (47.6- 242.7)	1.49 (0.73- 3.37)	24	68.0 (36.7- 104.8)	1.28 (0.62- 2.48)	not specified

[a] n is the number of participants that consumed any NNSs; [b] The 25th and 75th percentile could not be calculated due to many participants did not consume the NNSs.

3.2. Correlations

Table 3 shows the correlations of the estimates of NNSs and total NNSs intake between both methods. Correlations for all of 7 NNSs and total NNSs intake were statistically significant ($p < 0.05$). Spearman´s correlations coefficients were moderate to strong and ranged from 0.50 for acesulfame K to 0.83 for saccharin. In the case of cyclamate and D-tagatose, the correlations were not applied due to the very small sample that consumed those NNSs. The Lin´s CCC was used to assess the reliability between FFQ of NNSs and the 3-DR. Within the 7 NNSs and total NNSs assessed we found a moderate level of agreement for saccharin (CCC= 0.66; 95% CI: 0.58 to 0.74), while for aspartame there was not agreement ($p > 0.05$). This suggests that although the values of both methods are associated, the level of agreement was much poorer.

Table 3. Spearman correlation coefficient (rho) and Lin´s concordance correlation coefficient (CCC) between daily NNSs intake per kilogram of body weight and the average of 3-DR among pregnant women.

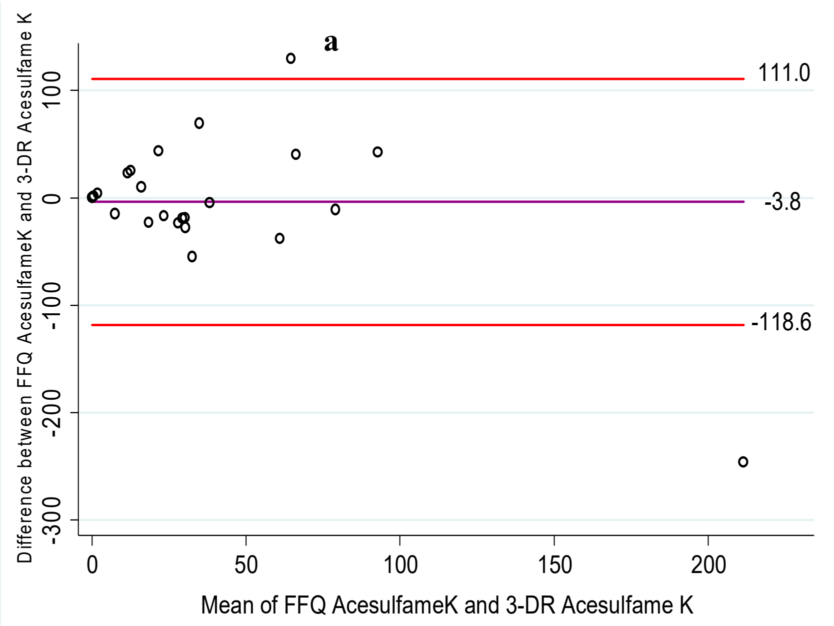
NNS type	Rho coefficients between FFQ and 3-DR	CCC between FFQ and 3-DR (95% CI)
Acesulfame K	0.50*	0.37 (0.07- 0.61)*
Aspartame	0.51*	0.28 (-0.01- 0.52)

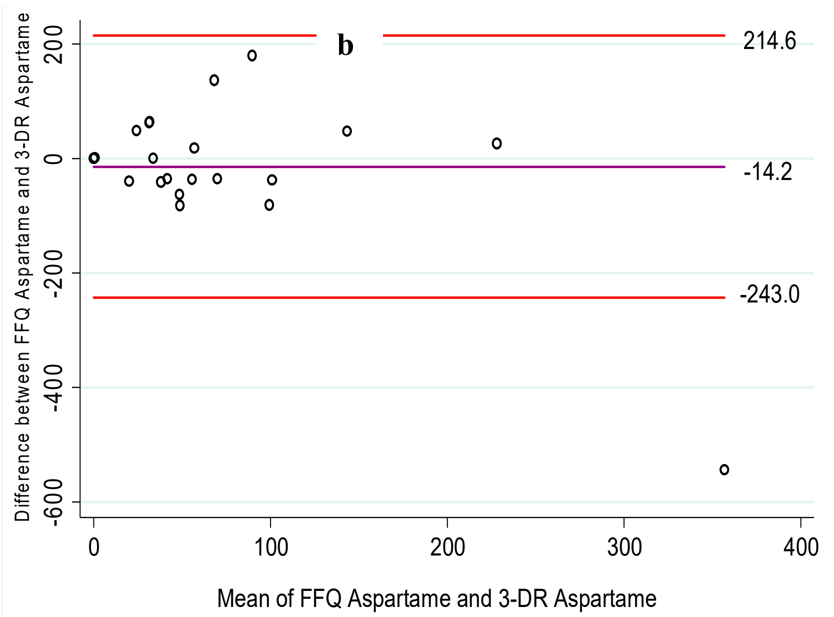
Cyclamate	n/a***	n/a***
Saccharin	0.83**	0.66 (0.58- 0.74)**
Sucralose	0.67**	0.35 (0.09- 0.56)**
Steviol glycosides	0.79*	0.22 (0.04- 0.38)
D-Tagatose	n/a***	n/a***
Total NNSs intake	0.66**	0.39 (0.02- 0.66)*

* p ≤ 0.05; ** p ≤ 0.01; ***n/a= not applied.

3.3. Agreement between methods

The Bland-Altman plots (Figure 2) measure the agreement of mg intake of total and each type of NNS between FFQ and 3-DR. Acesulfame K and aspartame were underestimated in the FFQ compared to the 3-DR, while other NNSs and total NNSs were overestimated. The mean differences in daily NNS intake were: -3.8 mg/day for acesulfame K, -14.2 mg/day for aspartame, 1.2 mg/day for saccharin, 19.9 mg/day for sucralose, 17.4 mg/day for steviol glycosides, and 54.8 mg/day for total NNSs.





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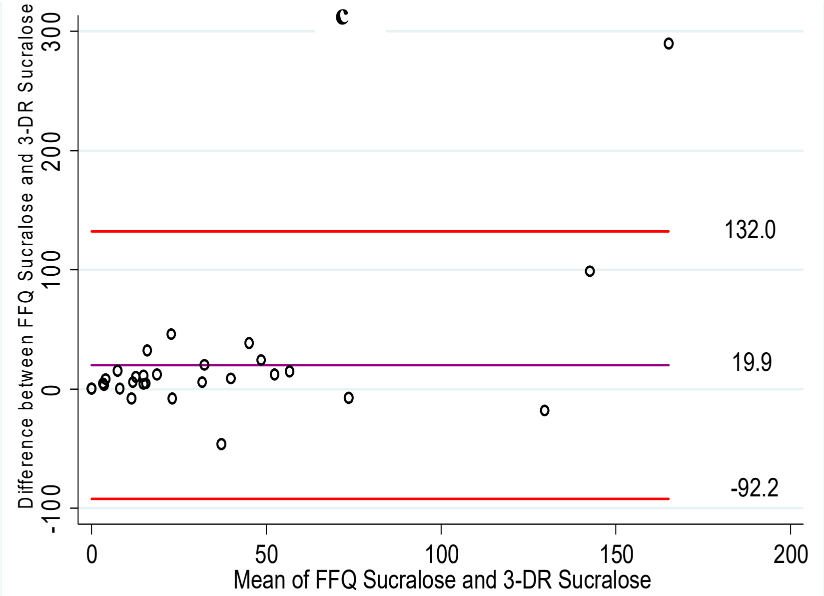

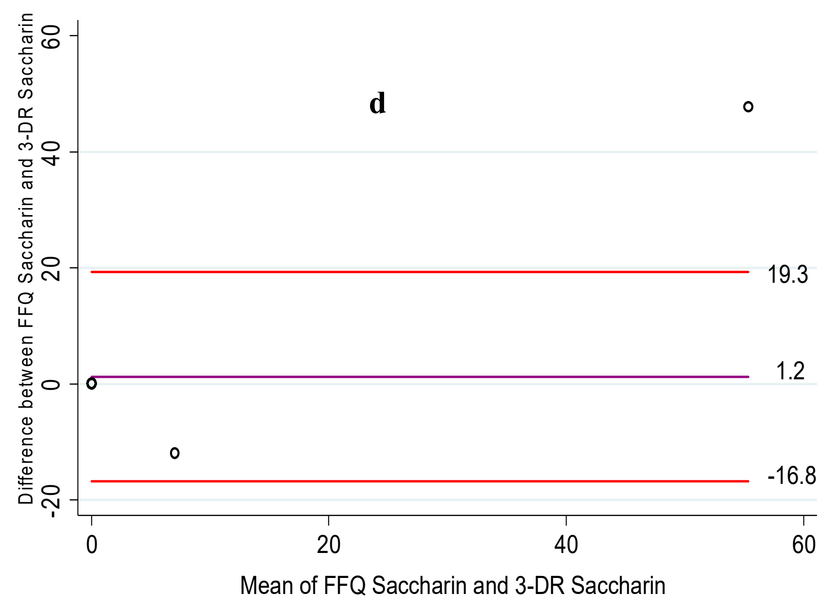

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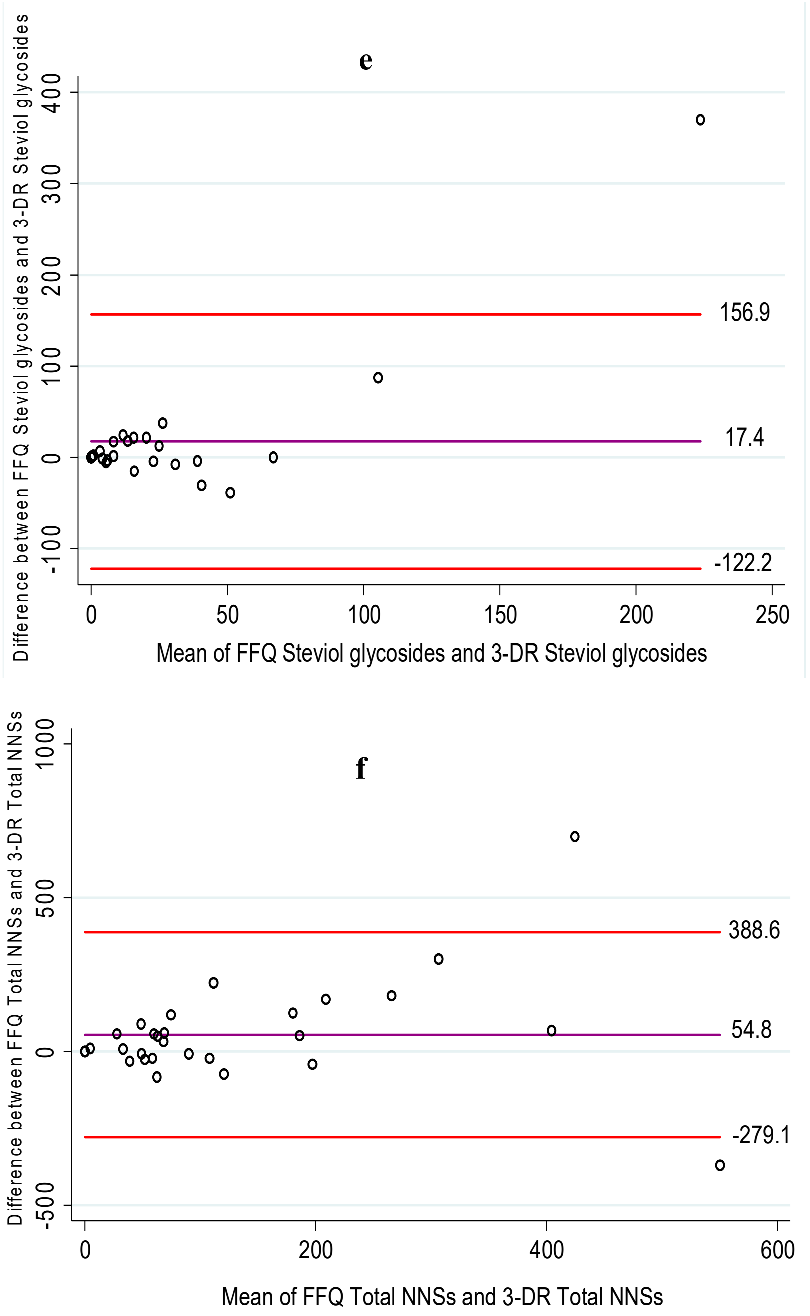

Figure 2. Bland-Altman plots assessing the agreement of daily NNSs intake (mg/day) from the FFQ of NNSs and the average of 3-DR. The center line represents the mean difference between the two methods and the upper and lower lines represent the 95% LoA for (a) acesulfame K; (b) aspartame; (c) saccharin; (d) sucralose; (e) steviol glycosides; and (f) Total NNSs intake.

4. Discussion

In this pilot study, we developed and assessed the validity of a FFQ to evaluate the intake of seven NNSs in pregnant women living in Santiago, Chile. To our knowledge, this is the first study to demonstrate the validity of a FFQ using a list of most food products containing NNSs available on the local market after the Chilean law on food labelling and advertising came into force and the subsequent reformulation of food and beverage products. Our results indicate that the FFQ of NNSs had fair-to-moderate validity. The Spearman´s correlation between FFQ of NNSs and 3-DR was >0.50 for most NNSs, three of them were above 0.60. For cyclamate and D-tagatose, we preferred to exclude the correlations as a large number of participants did not consume these NNS, and to avoid a false sense of relationship [42]. This is because only 1.3% of foods containing NSS in the Chilean market contained cyclamate [20]. In the case of D-tagatose, Article 146 of the Chilean food sanitary regulations states that the use of D-tagatose as a NNS is not allowed in foods intended for weight control diets, nor in foods that are sugar-free, or low or reduced in sugar or sugars (mono and disaccharides), calories and fat [43]. While tagatose was originally evaluated as a food additive, it is now considered a novel food in some countries such as the United States, Australia, New Zealand and the European Union [44].

A similar correlation was observed by Myers et al., who measured five types of NNSs (saccharin, aspartame, acesulfame potassium, sucralose and erythritol) in adults residing in southwest Virginia. The Spearman´s correlation between FFQ and three 24-hour dietary records ranged from 0.51 to 0.59 [28]. Although a direct comparison is not possible, due to differences in food classification and methods of assessing NNSs consumption, similar correlations were observed for acesulfame K and Aspartame. In addition, we observed higher correlations for saccharine and sucralose in our study. Overall, these values are similar to those reported in FFQ validation studies, which generally consider correlations between 0.4 and 0.7 to be valid [32,45].

Historically, the most common method for testing the validity of FFQs is the ICC. However, ICC is very sensitive to sample heterogeneity [46]. We incorporated Lin´s concordance coefficients to overcome these criticisms for assessing agreement for both methods and capitalizing that the "concord" command in Stata also provides Bland and Altman´s limits of agreement graphics [47]. Our results indicate a fair to moderate agreement, but with a proportional bias, i.e. where the differences between the methods tend to first reduce and then increase when measuring larger NNS intakes. The overall mean differences in total NNSs indicate that FFQ of NNSs tends to overestimate the total intake by 55 mg, which would be equivalent to 138 mL of diet soda. In the Chilean market, 100 mL of diet soda contains 24 mg of aspartame and 16 mg of acesulfame K. Moreover, we found an overestimation of saccharin, sucralose and steviol glycosides intake by the FFQ of NNSs compared with 3-DR, and an underestimation of acesulfame K and aspartame. These mean differences were larger than those reported by Myers et al, especially for sucralose and aspartame [28].

Previous studies that assessed NNS consumption in Chile have mainly focused on children [48–51], adults [52,53] and only one study has addressed pregnant women [54]. To evaluate NNS intake, different dietary instruments have been used such as the 24 hours' recall [51] or the FFQ [48,50,52,54]. The studies that have used FFQ have some shortcomings. Durán et al. designed a FFQ based on a list from 122 food products available in supermarkets. Nonetheless, it was not clear what type of validation was performed [48]. Similarly, Fuentealba et al. pointed out that their FFQ was validated by experts, but without further details [54]. On the other hand, Martínez et al. assessed NNS intake among 250 Chilean children aged 6-12 years using a FFQ originally designed for the US population [50]. Because of differences in dietary habits, the use of dietary instruments in other populations may not be an appropriate alternative and caution should be taken when interpreting the results [32,45]. In addition, the use of methods with limited validity may produce a regression dilution bias [55] that will lead to a serious underestimation of the associations between intake and disease in epidemiological studies [56].

Our results show that with both dietary instruments, more than three-quarters of pregnant women consumed NNSs daily, mainly sucralose, and none of them exceeded the acceptable daily intake for any of the NNSs assessed. This high prevalence of pregnant women reporting intentional intake of NNSs may be of concern. There is increasing evidence to suggest that pregnant women consume as much or even more NNSs as the general population [13]. This steady increase in consumption is probably due to increased awareness of the harmful effects of excessive sugar consumption [22], but also to the massive presence of NNSs in different food products. Indeed, we recently found that out of 1489 products available on the Chilean market, more than fifty percent contained at least one NNS, which is a particularly high proportion when compared to other countries [20]. However, few studies have been conducted on humans to elucidate the effects of NNS consumption during pregnancy and the postnatal period, as well as the long-term consequences on the offspring.

The strengths of this study are that the FFQ of NNSs is a dietary instrument that allows for quick and easy measurement of NNS consumption. In addition, it can differentiate between sources of NNSs, it is brand specific, and captures both beverage and food sources of NNSs, since the FFQ of NNSs was developed considering all possible sources of NNSs in the Chilean market. Nevertheless, our study has several limitations. Although more than one third of the Chilean population lives in Santiago, according to the National Statistics Institute (INE), this study was a convenience sample. Therefore, the participants' backgrounds do not necessarily reflect the pregnant women living in the country. Secondly, the education level of the participants in this study was higher than that of the general population [57]. Nevertheless, a higher level of education was associated with higher accuracy of responses to the FFQ and 3-DR [58]. As Chile is increasingly receiving immigrants, especially from Latin America and Caribbean countries, with an estimated number of foreign residents reaching 1,492,522 (approximately 10% of the population) [59], we included pregnant women from other nationalities such as Venezuela, Peru, Colombia and Ecuador. However, regional food and beverages items were not taken into account, so the FFQ likely underestimates NNS intake. In addition, our sample size was insufficient to conduct a sub-group analysis.

5. Conclusions

In conclusion, this pilot study demonstrated that, at least for the assessment of the consumption of some NNSs, the FFQ can be used in pregnant women living in Santiago, Chile. The FFQ of NNSs may be a useful method for future epidemiological studies on the nutritional status of pregnant women. However, it would be advisable to also consider biological markers as a reference method for future validation studies and to assess the validity of this FFQ in multiethnic populations.

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